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T20. P7R

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From: Saidha, Tekchand
Sent: Saturday, August 02, 2003 2:52 PM
To: STIC-ILL
Subject: art request - 09/837235

A copy of the following refrence(s) is requested :

1. ANSWER 6 OF 7 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1982:300038 BIOSIS
DOCUMENT NUMBER: BA74:72518
TITLE: OZONE INDUCED FORMATION OF O O' **DI TYROSINE** CROSS LINKS IN PROTEINS.
AUTHOR(S): VERWEIJ H; CHRISTIANSE K; VAN STEVENINCK J
CORPORATE SOURCE: SYLVIUS LAB., DEP. MED. BIOCHEMISTRY, WASSENAARSEWEG 72, 2333 AL LEIDEN.
SOURCE: **BIOCHIM BIOPHYS ACTA, (1982) 701 (2), 180-184.**

2. TITLE: CHEMICAL NATURE OF MONOGENEAN SCLERITES PART 1 STABILIZATION OF CLAMP **PROTEIN** BY FORMATION OF **DI TYROSINE.**
AUTHOR(S): RAMALINGAM K
SOURCE: PARASITOLOGY, (1973) 66 (1), 1-7.
CODEN: PARAAE. ISSN: 0031-1820.

TITLE: CD and proton NMR studies on the side-chain conformation of tyrosine derivatives and tyrosine residues in di- and tripeptides
AUTHOR(S): Juy, Michel; Lam Thanh Hung; Fermandjian, Serge
CORPORATE SOURCE: Dep. Biol., Cent. Nucl. Stud., Gif-sur-Yvette, 91191, Fr.
SOURCE: **International Journal of Peptide & Protein Research (1982), 20(4), 298-307**

3. Journal of the American Chemical Society (1985), 107(3), 659-66

4. **BIOCHEMICAL JOURNAL,** (2003 Mar 1) 370 (Pt 2) 729-35.

5. Salt-**stabilized protein** formulation

SOURCE: **Research Disclosure (1995), 370, 56-7**

Thank you !

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37013
Salt-stabilized Protein Formulation

A formulation of protein includes a stabilizing polyol such as glycerol or tris(hydroxymethyl)aminomethane. The formulation includes a physiologically compatible buffer, incorporated for maintaining the pH exhibited by the composition within a range in which the protein, for example, bovine somatotropin is bioactive. Generally, the pH exhibited by the solution should be between a minimum of about 4.5 or, about 5 (5.7) and a maximum of the greater of about 7 and about the isoelectric point of the somatotropin.

In order to promote wetting of the protein by the buffer/polyol excipient during preparation of the formulation, a wetting agent, such as a nonionic surfactant is incorporated as well. Such surfactant also inhibits foaming. The surfactant can be present in the excipient at amounts between about 0.005% and about 2.5%, for example, about 0.25%. A particular nonionic surfactant is a polyethoxylated sorbitan ester, such as a tri(polyoxyethylene) ester of sorbitan mono-oleate, such as that sold under the trade designation Tween 80 by ICI Americas Inc.

It is desirable that somatotropin does not precipitate or otherwise separate from the excipient, either on standing or under the influence of shear encountered in passage of the composition through the discharge opening of an infusion pump. The concentration of somatotropin in the composition may be at least about 10% by weight or at least about 15% by weight, or at least about 20% by weight or at least about 25% or even about 30% by weight. The somatotropin concentration may range as high as about 45% by weight. The polyol concentration may be at least about 20% by weight or 25% by weight and may range as high as 80% by weight or 70% by weight or 60% by weight or 50% by weight or 40% by weight. A relatively high glycerol content additionally provides a bacteriostatic effect. It is generally considered that an excipient containing about 50% glycerol provides bacteriostatic effect. The osmotic implant may further contain an estrogenic agent, for example, 17-β-estradiol, at a concentration of about .05 to about 1%, or about 0.18 to about 0.72%.

The formulation may further comprise a wetting agent, such as nonionic surfactant with optimum

concentrations between about 0.005% or about 2.5% by weight. Except for the buffer salt, which in the case of a phosphate buffer may typically comprise 4% to 7% by weight, and the sodium or potassium chloride which may be added to stabilize the formulation, described below, the balance of the formulation typically is water. A preferred formulation contains at least about 7% water, more preferably at least about 15% water, and even more preferably between about 25% and about 35% by weight water.

An alkaline halide such as sodium chloride or potassium chloride is added to the excipient prior to formulation with somatotropin. It has been found that this facilitates maintaining homogeneity of the formulation during filling of the implants, for example, when using a metal-associated somatotropin such as zinc-associated somatotropin. Following addition of the somatotropin to the excipient, the filled implant can be subjected to heat treatments from about 6 to 24 hours, for example, 16 hours, at a temperature between about 35°C and 50°C, for example 39-46°C (about 40°C). Preferably the alkaline chloride comprises about 1 to about 4% by weight of the final formulation.

The formulation is normally a clear, homogeneous single phase. The formulation appears as a solid or semisolid at a storage temperature of about 4°C. The formulation decreases in viscosity to produce a viscous liquid at the body temperature of an animal. In this way, the formulation is dispensable without being readily fluid at all temperatures.

For an example, the formulation may be an aqueous suspension of somatotropin formulated as herein described, including glycerol, monobasic and dibasic sodium phosphate buffer, Tween-80, an alkaline halide salt such as sodium chloride and/or potassium chloride, but are not being limited to these ingredients, in addition to the active ingredients such as bST and the estrogenic agent.

One formulation comprises 36.5% ± 1.5% Zn-bST in a phosphate buffer, glycerols, wetting agent, salt excipient blend where the w/w/w/w proportions of phosphate buffer, glycerol, Tween-80, and KCl are 48.38/48.38/0.24/3.0 respectively. The phosphate buffer is 60:40 monobasic:dibasic sodium phosphate, and the molarity is 0.45. The composition may also contain an estrogenic agent comprising about 0.06% to about 3.0% 17-beta-estradiol.

Disclosed Anonymously
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